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Distribution and Concentration of Major Soluble Carbohydrates in Hevea Latex, the Effects of Ethephon Stimulation and the Possible Role of these Carbohydrates in Latex Flow

F.C. LOW

The distribution and concentration of sugar and total cyclitols in high, medium and low-plugging clones of Hevea *were examined. The high-plugging clone (Tjir 1) was shown to contain significantly higher total cyclitols than the low-plugging clone (RRIM 501). However, the sucrose content of the high-plugging clone was significantly lower than that in the low-plugging clone. Total cyclitols and sucrose seemed to be confined mainly in the C serum whilst glucose was mainly in the B serum. Ethephon stimulation significantly reduced total cyclitol and sucrose concentrations in latex serum but not the glucose concentration.*

The possible significance of these findings in relation to latex flow is discussed.

Quebrachitol (1-0-methyl-*l*-inositol) is the most abundant carbon compound in *Hevea* latex after rubber. Its amount varies from one clonal latex to another and is usually in the order of 1% by weight of latex[1]. Smith[2] first demonstrated that *Hevea* latex contained *l*- and *m*-inositols in addition to quebrachitol. Bealing and Chua[3] reported that quebrachitol was the predominant cyclitol in latex while bark juice contained approximately equal proportions of quebrachitol and *l*-inositol. There was also a preliminary report[4] that quebrachitol was located mainly in the serum phase of latex and that it might contribute substantially to the total osmotic pressure of latex.

Sucrose is the most predominant sugar in *Hevea* latex. Since it is the major sugar in latex, its distribution between the latex phases, changes induced by ethephon stimulation and possible role in latex flow were examined in the present study.

MATERIALS AND METHODS

Experimental Material

Experiment 1. Six trees from each of the clones Tjir 1, RRIM 600 and RRIM 501 were selected from Field 14D at the RRIM Experiment Station, Sungei Buloh. All these trees were fourteen to fifteen years old and were tapped on *Panel B* in a downward direction on the S/2.d/2 system. Latex was collected by the method described by Moir[5]. Latex was collected in ice-cooled vessels for 30 min after tapping. Latex samples within each clone were pooled and centrifuged for 60 min with minimum delay in a refrigerated Spinco Model L2-65B ultracentrifuge, using the No. 21 rotor at 21 000 r.p.m. (g maximum 59 000). Under these conditions latex was separated into three main fractions[5,6] — a cream of rubber at the top of the tube, a clear cytosol of latex (C serum) in the middle and a sediment (bottom fraction). The C serum thus obtained was collected by puncturing the centrifuge tube.

B serum was prepared from the same latex samples as the C serum. Bottom fractions pooled within each clone were washed three times by resuspension in 0.3M phosphate−KCl buffer at pH 7.0 followed by centrifugation. After repeated freezing and thawing of the washed bottom fractions, a

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clear B serum was obtained after centrifugation at 40 000 r.p.m. in rotor 40 (g maximum 145 000) for 45 min in a refrigerated Spinco ultracentrifuge.

Experiment 2. Sixteen trees from each of the clones Tjir 1, GT 1, PB 86, RRIM 600 and RRIM 501 were selected from another plot in Field 14D. All these trees were of the same age and tapping system as those in *Experiment 1.* The selected trees in each clone were divided into two groups. One group was treated with 10% a.i. ethephon in palm oil applied as a 3.8 cm band below the tapping cut on lightly scraped bark. The control trees were similarly scraped and applied with palm oil without stimulant. The trees which had been stimulated repeatedly for about three years when this experiment was initiated by Yip and Gomez[7] were stimulated at two-monthly intervals.

Frequency of Sampling

Sampling was irregular at the initial stage of the experiment, especially in *Experiment 1.* However, in *Experiment 2* sampling was usually performed twice a month. Sampling was performed for a period of one year in both experiments.

Estimation of Total Cyclitols

Quebrachitol, *l*- and *m*-inositols were estimated by the method modified from Bealing[8] as described in the *appendix.*

Estimation of Reducing Sugar (Glucose)

Reducing sugar was measured by the Nelson-Somogyi method[9,10]. The amounts of reducing sugar present were calculated by comparison with standard solutions containing $0-0.5 \times 10^{-6}$ moles glucose.

Estimation of Sucrose

Sucrose was estimated as reducing sugar produced by hydrolysis. Samples were estimated for reducing sugar before and after acid hydrolysis, and the difference between the latter and the former was a measure of sucrose.

RESULTS

Distribution and Concentration of Total Cyclitols

The total cyclitol concentrations in the B and C sera of latex are shown in *Figure 1.* In general, C serum had a significantly higher total cyclitol concentration than B serum *(Figure 1* and *Table 1).* In both *Experiments 1* and *2,* Tjir 1 was shown to contain a significantly higher total cyclitol concentration than RRIM 501 *(Tables 1* and *3).* The total cyclitol concentration of RRIM 600 was similar to that of Tjir 1 in its C serum and to that of RRIM 501 in its B serum *(Figure 1).* Significant differences in total cyclitol concentration between clones and between B and C sera were found *(Tables 2* and *4).*

Effects of Ethephon Stimulation on Total Cyclitol Concentration

Ethephon stimulation significantly reduced the total cyclitol concentration in C serum *(Figure 2* and *Tables 3* and *4).* Of the five clones investigated in *Experiment 2,* the decrease in total cyclitol concentration was 14% to 28% that of control. Although, day-to-day variations in total cyclitol levels were observed in both the control and stimulated trees, these effects were insignificant. The depression in total cyclitol levels on stimulation was consistent (with a few exceptions, *i.e.,* three samples in RRIM 501, one each in RRIM 600 and GT 1 and two samples in PB 86 out of a total of twenty-six samples) throughout the one year of sampling.

Distribution and Concentration of Glucose

In *Experiment 1,* B serum was found to contain a significantly higher glucose concen-

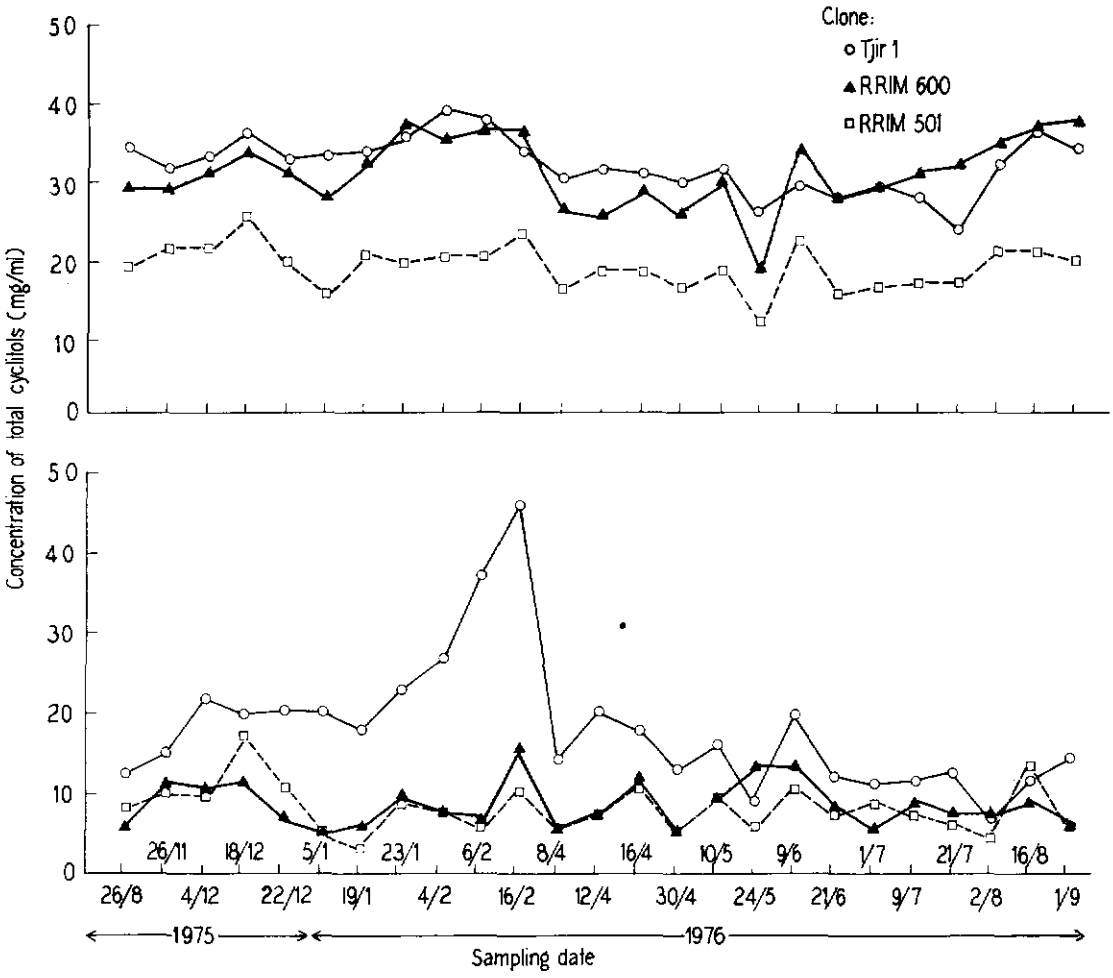

Figure 1. Total cyclitol concentration in C serum (top) and B serum (bottom).

TABLE 1. MAJOR SOLUBLE CARBOHYDRATES IN LATEX SERA[a]

Clone	Total cyclitols (mg/ml)		Glucose (mg/ml)		Sucrose (mg/ml)	
	B serum	*C serum*	*B serum*	*C serum*	*B serum*	*C serum*
RRIM 501	8.23	18.83	1.66	0.65	5.21	10.48
RRIM 600	8.51	31.59	1.35	0.69	2.09	5.75
Tjir 1	18.69	31.94	1.71	0.48	2.59	4.14
S.E.	± 0.96		± 0.09		± 0.50	
L.S.D.	2.66		0.24		1.38	
Mean	11.81	27.45	1.57	0.61	3.29	6.79
S.E.	± 0.31		± 0.05		± 0.29	
L.S.D.	0.86		0.14		0.80	

[a] Average of twenty-five readings

TABLE 2. MEAN SQUARES OF ANALYSIS

Source	df	Total cyclitols	Glucose	Sucrose
Serum	1	9 173***	35***	459***
Clones	2	1 743***	0.216 NS	298***
Serum × clones	2	541***	1.018**	20*
Error	144	23	0.183	6
Mean		19.63	1.09	5.04
S.D.		4.80	0.43	2.50
C.V. (%)		24.5	39.2	49.5

*P < 0.05 **P < 0.01 ***P < 0.001
NS Not significant

tration than C serum *(Tables 1* and *2)*. The C serum glucose concentration was significantly different between clones in *Experiment 2 (Table 4)* but not in *Experiment 1 (Table 2)*. The reason for the discrepancy is not clearly understood at present.

Effects of Ethephon Stimulation on Glucose Concentration

Ethephon stimulation did not alter the C serum glucose concentration *(Table 4)*.

Distribution and Concentration of Sucrose

Significant clonal differences in sucrose concentration were found in all three clones in *Experiment 1* and three out of the five clones in *Experiment 2*. RRIM 501 was found to contain a significantly higher sucrose concentration than Tjir 1 in both its B and C sera *(Figure 3* and *Table 1)*. In all the three clones examined in *Experiment 1*, C serum had a significantly higher sucrose concentration than B serum. Often, the C serum sucrose concentration was about twice that of its B serum *(Table 1)*.

Effects of Ethephon Stimulation on Sucrose Concentration

Ethephon stimulation significantly reduced the overall mean of the C serum sucrose concentration. Considering individual clones, stimulation has not significantly decreased sucrose concentration for Tjir 1 and PB 86. For the other three clones the reduction in sucrose concentration was 10%—30% *(Figure 4* and *Tables 3* and *4)*.

DISCUSSION

Distribution of the Major Soluble Carbohydrates between Latex Phases

The major soluble carbohydrates in latex are total cyclitols, sucrose and glucose in that order. C serum seemed to contain most of the major soluble carbohydrates, namely total cyclitols and sucrose, whilst glucose was distributed mainly in the lutoids with lesser amounts of it in the C serum. The finding that sucrose and total cyclitols were located mainly in the serum phase of latex was hardly surprising since the enzymes for carbohydrate metabolism were present in the C serum [8,11]. The presence of these major soluble carbohydrates in the C serum strongly suggests their osmotic role in relation to latex flow. However, the high concentration of glucose in lutoids instead of serum is not clearly understood.

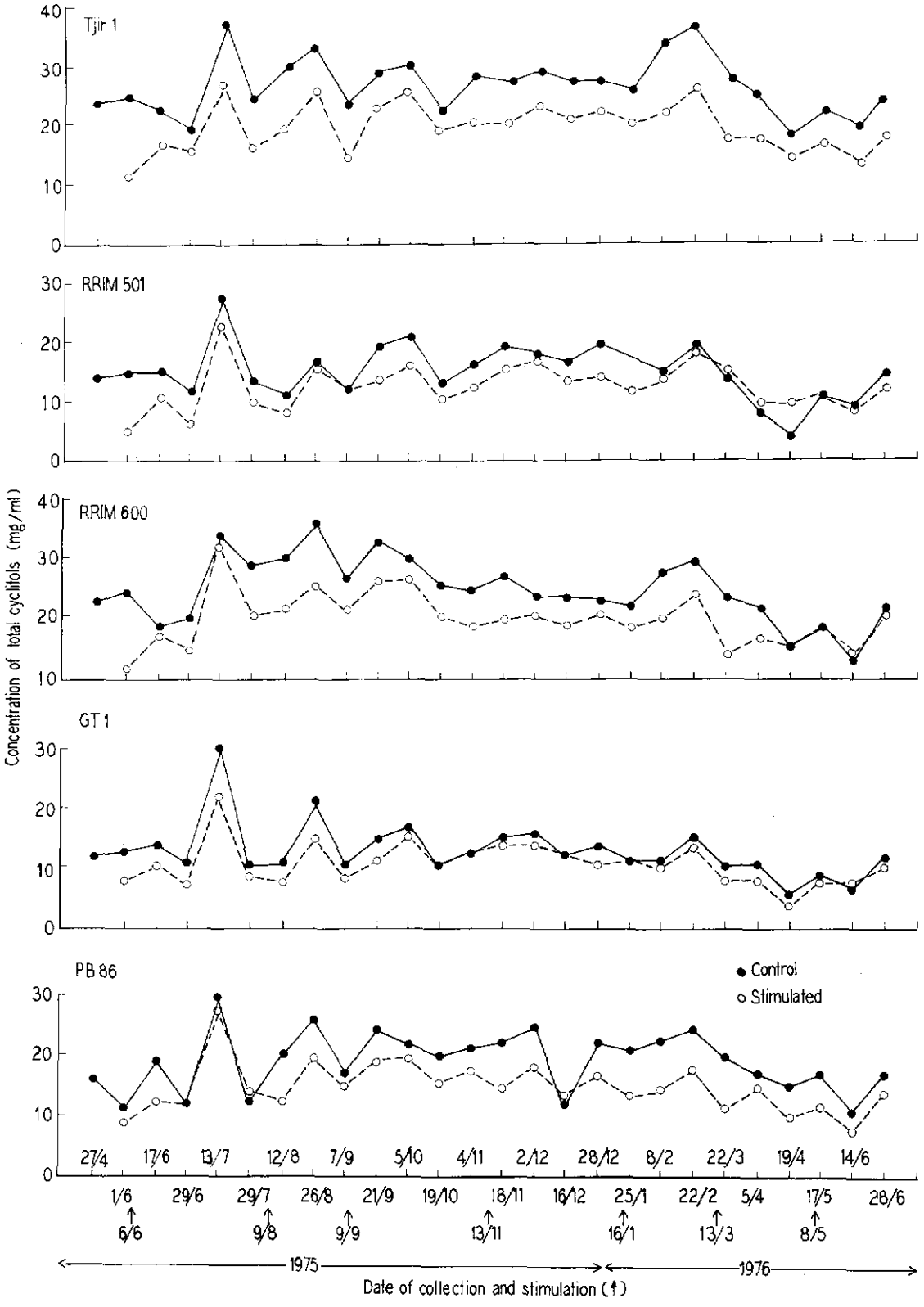

Figure 2. Effects of ethephon stimulation on C serum total cyclitol concentration.

TABLE 3 TABLE OF MEANS OF THE CONCENTRATION OF TOTAL CYCLITOLS AND SUGARS[a]

Clone	Total cyclitols (mg/ml serum)						Glucose (mg ml serum)						Sucrose (mg/ml serum)					
	Tjir 1	PB 86	RRIM 600	GT 1	RRIM 501	Mean	Tjir 1	PB 86	RRIM 600	GT 1	RRIM 501	Mean	Tjir 1	PB 86	RRIM 600	GT 1	RRIM 501	Mean
Control	26 54	19 26	25 71	13 04	14 81	19 87	0 580	0 532	0 772	0 696	0 711	0 659	6 77	7 60	6 23	7 88	10 15	7 73
Stimulated	19 24	15 29	20 91	11 03	12 75	15 84	0 634	0 561	0 783	0 665	0 738	0 676	6 25	6 95	4 37	5 35	8 04	6 19
S E	± 0 4762					± 0 2130	± 0 02					± 0 0089	± 0 3955					± 0 1769
L S D	1 3201					0 5903	0 055					0 0248	1 0962					0 4902
Mean	22 89	17 28	23 31	12 04	13 78	17 86	0 607	0 547	0 779	0 681	0 725	0 668	6 51	7 28	5 30	6 62	9 10	6 96
S E	+ 0 3367						± 0 0141						± 0 2796					
L S D	0 9334						0 0392						0 7751					

[a]Average of twenty five readings

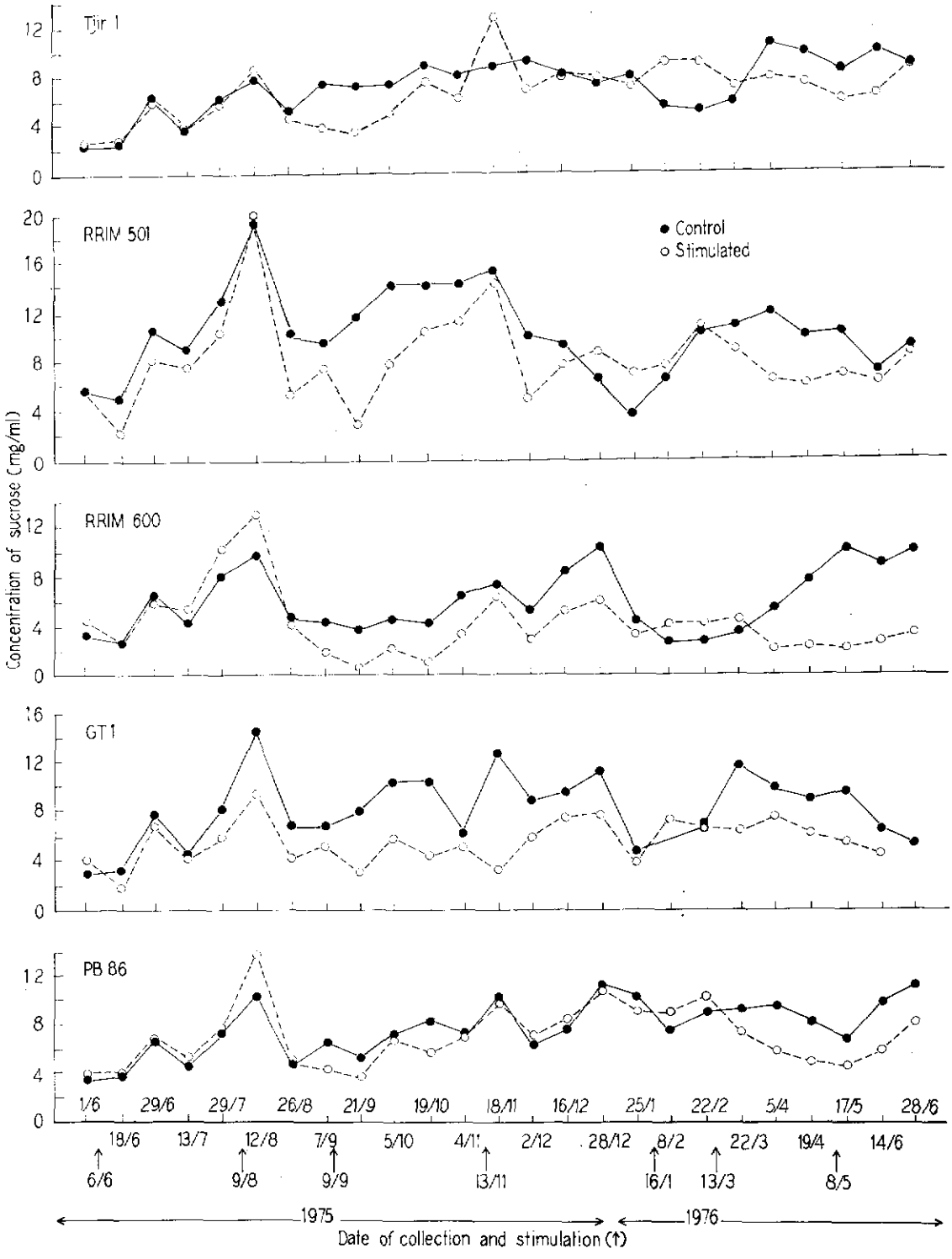

Figure 4. Effects of ethephon stimulation on C serum sucrose concentration.

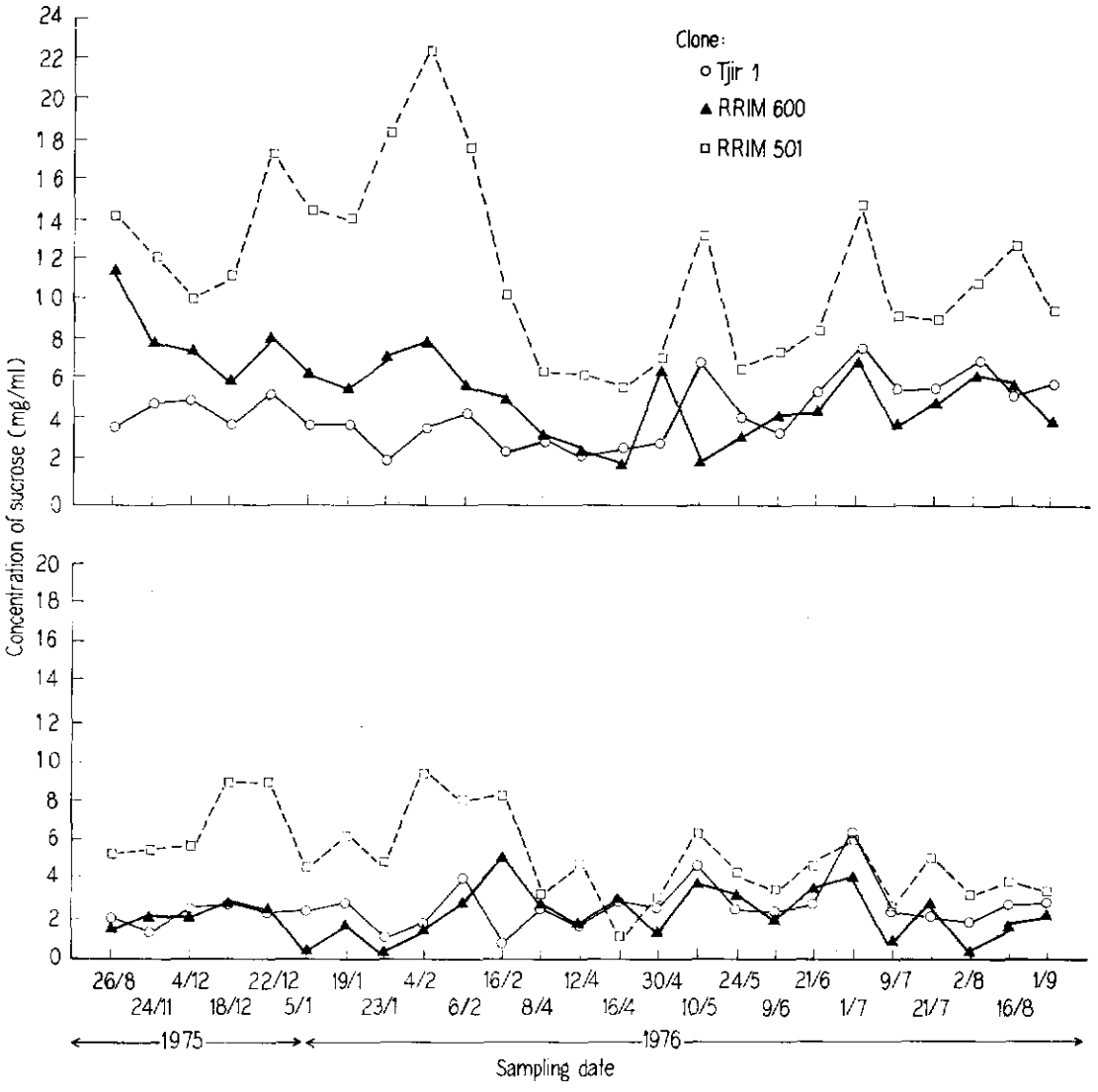

Figure 3. Sucrose concentration in C serum (top) and B serum (bottom).

TABLE 4. ANALYSIS OF VARIANCE

Source	d.f.	Mean square		
		Total cyclitols	Free reducing sugar	Sucrose
Days	24	168.67	0.249	36.18
Clones	4	1 323.70***	0.426***	96.66***
Treatments	1	1 013.04***	0.019 NS	147.23***
Clones × treatments	4	60.01***	0.013 NS	10.17**
Error	216	5.67	0.010	3.91

*P<0.05 **P<0.01 ***P<0.001

NS Not significant

Effects of Ethephon Stimulation on the Major Soluble Carbohydrates

Total cyclitols. Ethephon stimulation resulted in a significant reduction in total cyclitols concentration in C serum. The decrease in the absolute value of total cyclitol concentration was highest in Tjir 1, followed by RRIM 600, PB 86, RRIM 501 and GT 1. This order of response to stimulation is somewhat similar to that for the plugging indices of these clones, as determined by Yip and Gomez[7] and Yip[12]. The decline in total cyclitol concentration with stimulation as observed in *Experiment 2* was 14% − 28% as compared to the fall in total solids (5% − 16%) recorded concurrently from the same trees[13]. The greater decline in total cyclitol concentration as compared to total solids content suggested that the fall in total cyclitol concentration on stimulation was not entirely due to dilution, nor was it a reflection of depletion of this carbohydrate in the tree. It was suggested that the apparent decrease in total cyclitol level with overtapping was a consequence of a decreased production of quebrachitol and a corresponding decline in metabolic efficiency[3]. It may be for these same reasons that the total cyclitol concentration is lowered following stimulation.

Sucrose. The significant depression of sucrose in latex serum after stimulation confirms other earlier reports[14,15]. In a preliminary experiment with trees which had never been stimulated, a transient increase in C serum sucrose concentration was observed immediately after stimulation[16], followed by a continued depression of sucrose concentration even several months after stimulation. The immediate effect on sucrose was a recent *single* observation and needs confirmation. The sustained depression in sucrose concentration with repeated stimulation had been observed earlier[14] and confirmed recently. However, the extent of depression in sucrose levels with stimulation varied from clone to clone. All clones were not similarly depressed in their clone means.

The fall in sucrose concentration following stimulation was probably caused by an enhanced invertase activity[17,18] and a diminished entry of sucrose from the surrounding cells into latex vessels[3]. The 10% − 30% decrease in sucrose concentration could not be caused by dilution alone. In another separate experiment[16], the serum sucrose concentration in stimulated trees was much lower than that of control trees even though the stimulated trees were yielding less than the control. Hence the serum sucrose concentration could be lowered on stimulation even though there was no increase in yield and therefore no dilution.

Glucose. Glucose concentration was unaffected by stimulation. The reason for this is not clearly understood at present.

Relationship of the Major Soluble Carbohydrates to Flow

Total cyclitols. It is interesting to compare the total cyclitol concentration of latex sera with the plugging indices of the clones studied. in *Experiment 1,* Tjir 1, which has a high plugging index[19], has significantly more total cyclitols in both B and C sera than RRIM 501, a low-plugging clone. On the other hand, RRIM 600, which has an intermediate plugging index, has a similar total cyclitol concentration in the C serum as Tjir 1, whilst its B serum total cyclitol concentration was nearly that of RRIM 501. In *Experiment 2,* Tjir 1, the highest plugging clone, was again shown to contain the highest total cyclitol concentration followed by RRIM 600, PB 86, RRIM 501 and GT 1. It is interesting to note that the order of total cyclitol concentration of these five clones is similar to the order of plugging indices[7,12] determined from the same trees of these clones *(Table 5).*

Because of its high concentration in latex (1% − 2%), total cyclitols have been suggested to have an osmotic effect in latex flow[4]. Since

TABLE 5. COMPARISON OF TOTAL CYCLITOL AND SUCROSE CONCENTRATIONS WITH AEROSOL OT STABILITY AND PLUGGING INDICES

Clone	Aerosol OT stability index*	Plugging index*	Total cyclitol concentration (mg/ml serum)	Sucrose concentration (mg/ml serum)
Tjir 1	3.08	5.66	26.54	6.77
PB 86	4.50	3.94	19.26	7.60
RRIM 600	4.19	3.45	25.71	6.23
GT 1	6.25	1.58	13.04	7.88
RRIM 501	7.66	2.60	14.81	10.15
S.E. ±	0.18	0.25	0.2130	0.1769
L.S.D.	0.50	0.69	0.5903	0.4902

*YIP, E. (1976)[12]. See also YIP AND GOMEZ (1975)[7]
Stability index = 135 determinations
Plugging index = 77 determinations

total cyclitols are the major soluble carbohydrates in latex serum, their contribution to latex osmolarity must be substantial. Indeed, quebrachitol has been reported to contribute as much as over 30% of the total osmotic pressure of latex serum[4]. A high total cyclitol concentration probably leads to a faster and higher dilution reaction during latex flow, resulting in possibly greater lutoid damage, a faster plugging reaction[20] and an earlier cessation of flow. Similarly, clones with low cyclitol content probably experience a slower dilution reaction, less lutoid damage, a slower plugging reaction and consequently a more prolonged latex flow.

Sucrose. Highly significant clonal differences in sucrose levels had been observed in *Experiment 1 (Table 2)* and three of the five clones in *Experiment 2 (Tables 3* and *4).* RRIM 501, which had a low-plugging index, had more sucrose in both B and C sera than RRIM 600 and Tjir 1. In *Experiment 2,* RRIM 501 was again shown to contain the most C serum sucrose followed by GT 1, PB 86, Tjir 1 and RRIM 600. This order of sucrose content in the five clones is nearly in the same order as that of their Aerosol OT stability indices established for these clones[7,12] *(Table 5).* As far as the five clones studied are concerned, a high-plugging clone seemed to contain high total cyclitols and low sucrose and *vice versa.* It remains to be established whether total cyclitol content is inversely related to sucrose levels, as was plugging indices inversely related to stability indices[7,12].

Currently, the role of sucrose in latex stability is not clearly understood. Since sucrose is a major soluble sugar in latex, it is probable that it contributes to the osmolarity of latex. However, the level of sucrose in serum is small compared to that of total cyclitols. Hence, the osmotic role of sucrose in relation to latex flow must similarly be minor compared to that of total cyclitols in general, and quebrachitol in particular.

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Rubber Research Institute of Malaysia
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APPENDIX

An Improved Method for the Estimation of Total Cyclitols

The analytical method used by Bealing[1] for the estimation of total cyclitols was adopted from a method[2] designed for animal tissues which do not contain sucrose. The method depended on the destruction of sugars (hexoses) by heating with barium hydroxide and then estimating the polyhydroxy compounds (*i.e.* the total cyclitols) by their reaction with periodate. Sucrose is relatively stable to alkali and paper chromatography confirmed that it was still present in the C serum after the barium hydroxide treatment, along with the cyclitols. The problem of sucrose interference was effectively overcome by the introduction of an acid hydrolysis step in the analytical procedure.

A 5 ml sample of C serum was acidified with one or two drops of concentrated acid and heated for 10—15 min in a boiling water bath.

Denatured proteins were removed by centrifugation and the clear supernatant was then estimated for total cyclitols [1]. However, because the removal of sucrose was complete, the preliminary chromatography of individual cyclitols prior to periodate oxidation described in the earlier method[1] was omitted. Since the increase in volume change was negligible, the volume change was neglected in the final calculations.

The successful removal of sucrose from C serum was confirmed by paper chromatography in ethyl acetate : n-butanol : acetic acid : water (8:3:3:2 v/v). Samples of C serum, sugar and cyclitol standards were chromatographed similarly for 24 hours. The success of the chromatography was assessed by colour development of the paper chromatograms with diphenylamine-aniline reagent [3].

The complete elimination of sucrose in the preliminary treatment by acid hydrolysis therefore gives a more accurate measurement of total cyclitol concentration in latex. Because the preliminary chromatography of individual cyclitols prior to periodate oxidation was avoided, the improved method is also less time consuming.

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